

Functional adaptations of *Begonia oxysperma* A. DC. and *Begonia ramosii* Merr. (Begoniaceae) revealed through morpho-anatomical analyses

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Abstract

Populations of *Begonia* occur along the altitudinal gradients of Mt. Banahaw de Lucban. *Begonia ramosii* Merr. populations occur at lower altitudes and are gradually replaced by *Begonia oxysperma* A. DC. at higher elevations. This suggests the possibility of local adaptations and phenotype localization. Populations of *Begonia* were assessed and examined for phenotypic variability to provide information about habitat-driven traits and functional adaptations. Analysis of functional traits in 19 populations of *B. oxysperma* and 8 populations of *B. ramosii* using morpho-anatomical techniques confirmed these hypotheses. Phenolic vacuolar inclusions, anthocyanin pigmentation in young leaves and stems, presence of medullary vascular bundles and secondary growth, chlorophyll *a* concentration, trichome density in vegetative parts and stomatal density were observed to vary either intraspecifically or interspecifically which suggest functional adaptation. These preliminary results provide an interesting avenue for plant evolutionary and ecological studies using *Begonia* as a model plant species.

Keywords: adaptation, environmental gradient, habitat-driven traits, Mt. Banahaw, variation

Introduction

The importance of microhabitat of a species is widely recognized in a variety of ecological phenomena. In response to several environmental conditions, many species of plants exhibit wide ranges of phenotypic variability. These variations in phenotypic expression are ecologically important and are key components of species adaptation and persistence. Functional traits are those properties of an organism or a part of an organism that strongly influence fitness through their effects on growth, reproduction and survival (Shah *et al.*, 2019). In plants, phenotypes expressed differently along environmental gradients as a result of adaptive mechanisms are termed localized phenotypes, whereas geographically unstructured phenotypes that occur throughout the range of the species are called generalized phenotypes (Bradshaw, 1965; Schlichting & Smith, 2002). Environmental heterogeneity is one of the factors that play a major role in the occurrence of such phenotypes

demonstrating versatility and diversity of the response of organisms to environmental changes. The occurrence of localized phenotypes among closely related taxa along environmental gradients frequently results in speciation, generating diverse group of organisms (Abbott, 2017; Fitzpatrick, 2012).

Begonia is one of the megadiverse groups of plants in the Philippines with 104 recorded endemic species (Hughes, 2008). Several species such as *Begonia oxysperma* A. DC. and *B. ramosii* Merr. occur along the altitudinal gradients of Mt. Banahaw de Lucban from the vicinity of the lowlands up to its peak. *B. oxysperma* belongs to sect. *Baryandra* A. DC., which has its center of diversity in the Philippines (Rubite *et al.*, 2013). Species in this section are rhizomatous, stemless, monoecious herbs, often lithophytic and associated with riverine habitats and waterfalls (Hughes *et al.*, 2018). On the other hand, *B. ramosii* belongs to sect. *Petermannia* (Klotzsch) A. DC. This section tends to be humicolous and occurs in microhabitats with more constant water availability (Rubite, 2013). These two species though belonging to a single genus, possess apparent morphological and anatomical differences and occur along specific altitudinal ranges. Their variations might have been facilitated by phenotypic plasticity or genetic differences which ultimately led to functional adaptations.

Altitudinal gradient is one of the factors that allows morpho-anatomical adaptations resulting to occurrence of

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localized phenotypes or may facilitate the persistence of generalized phenotypes. Körner (2007) stated that altitudinal gradients provide an ideal experimental opportunity for studying the variation of functional traits in plants in response to environmental factors. Mt. Banahaw de Lucban, having a maximum altitude of 1875 meters above sea level (masl) is an ideal system in which to uncover the role of environment in shaping inter- and intraspecific variation and functional adaptation of plants such as *Begonia*. Several studies regarding the distribution and diversity of plant and animal species have been conducted along the altitudinal gradients of Mt. Banahaw (Almazol & Quintana, 2009; Banaticla & Buot, 2005; Gascon *et al.*, 2013; Santiago & Buot, 2015; de Guia *et al.*, 2018) but the extent by which environmental gradients affect the distributional limits and the occurrence of certain phenotypes have not yet been documented. Moreover, many studies about inter- and intraspecific variations on heterogeneous environment had been conducted (Bosabalidis *et al.*, 2003; de Bello *et al.*, 2015; Yuliani *et al.*, 2015) but not explicitly in the context of *Begonia* species. Thus, this paper investigated the functional adaptations of *B. oxysperma* and *B. ramosii* along the altitudinal gradients of Mt. Banahaw de Lucban, Philippines using morpho-anatomical techniques.

Materials and Methods

Study site

This study was conducted in the northeastern slope of Mount Banahaw de Lucban (Figure 1) which lies between 13° 55' and 14°10' N latitude and 121°26' and 121°35' E longitude. Its peak has an elevation of 1875 masl. It is a protected landscape and among the most important ecosystems in the Philippines serving as habitat for several organisms including numerous endemic species.

Collection of plant samples

Collection permits were secured from Southern Luzon State University and Department of Environment and Natural Resources (Permit No. 2016-043 LQ; Permit No. 2016-037 LQ) before plant collection. The plant samples were collected from 700 masl up to 1200 masl. A distance of 150 m on both the left and right sides of the trail was surveyed for the presence of the target species. Fresh, whole plant samples of *B. oxysperma* and *B. ramosii* were collected from the sampling sites. Due to restriction in the collection of plant samples, at least three samples were collected from a population with greater than 10 individuals.

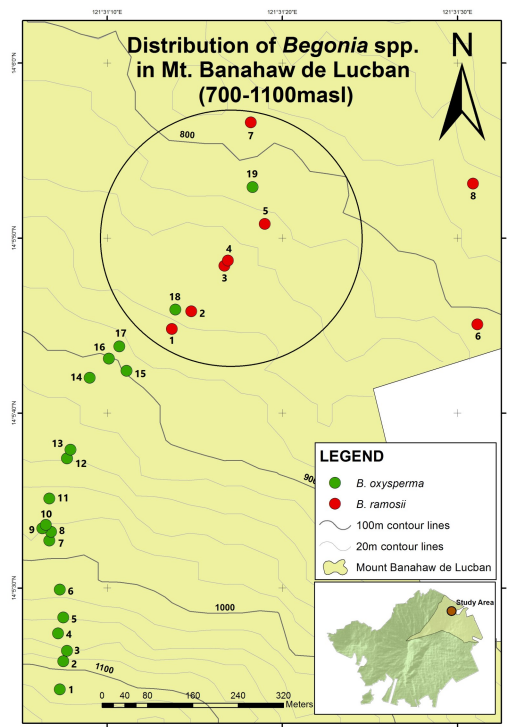


Figure 1. Topographical map showing the distribution of *Begonia* spp. along the altitudinal gradients of Mt. Banahaw de Lucban. The overlapping population of *B. ramosii* and *B. oxysperma* is indicated by a circle.

Morpho-anatomical examination

The morphology of the vegetative and reproductive parts of *Begonia* including the quantitative and qualitative characters was examined *in situ* and in the laboratory. The plant parts were photographed for documentation purposes. A total of 30 tissue sections were used to examine the morpho-anatomy of the species.

Using paraffin and freehand sectioning methods, the anatomy of the vegetative and reproductive parts of the *Begonia* species was investigated. The standard protocols for plant anatomical investigation such as dehydration, staining, mounting, and microscopy was followed (Robil & Tolentino, 2016; Ruzni, 1999; Tolentino, 1995). The stomatal density was investigated by observation of paradermal sections while the trichome density and morphology were observed directly under the microscope (Ken-a-vision) and then photographed. The distribution of stomata was categorized as single or clustered. The anatomical characteristics of the stomata, guard cells and subsidiary cells were also described.

For the determination of chlorophyll *a* concentration, 5 g of leaf lamina obtained from the third leaf relative to the terminal leaf bud was extracted manually in a mortar and pestle

Table 1. Morpho-anatomical comparison of *Begonia ramosii* and *Begonia oxysperma*.

	<i>Begonia ramosii</i>	<i>Begonia oxysperma</i>
Morphological characters		
(a) vegetative structures	sub-shrub; erect and green or maroon stem	epiphytic vine; succulent stem; no apparent anthocyanin pigmentation in any vegetative parts except for the lamina of young leaf
	petiole has evenly distributed minute trichomes	petiole has multicellular, non-glandular trichomes
(b) reproductive structures	monoecious	monoecious
	dichasial cyme inflorescence	multiparous cyme inflorescence
(b1) carpellate flower	has five pink tepals with deep pink pigmentation at the base	not described because all the carpellate flowers had already developed into fruits
	carpels are actinomorphic, yellow in color, and have three styles associated with bifid spiraling stigmas	
	three-winged inferior ovary	three-winged inferior ovary
(b2) staminate flower	has two tepals which are white in color with deep pink spot at the base	has four tepals, with the outer pair being orange in color and the inner pair being pale orange
(b3) fruit	dry dehiscent capsules with three wings; pale pink to green in color	dry dehiscent capsules with three wings; dark orange to brown in color
(b4) seed	dark brown and rounded; 0.06 to 0.10 mm	dark brown, elongated; 0.12 to 0.20mm
Anatomical characters		
(a) vegetative structures	uniseriate epidermis	uniseriate epidermis
(a1) stem	transverse section has ridges and furrows	transverse section is circular in shape
	internal cortex (3 to 5 layers of parenchyma cells)	internal cortex (5 to 15 layers of parenchyma cells)
	external cortex (3 to 5 layers of collenchyma cells)	external cortex (4 to 7 layers of collenchyma cells)
	fascicular vascular bundles	fascicular vascular bundles
	prominent interfascicular region consisting of purple, rectangular-shaped	less prominent interfascicular cambium

Table 1 (cont.) Morpho-anatomical comparison of *Begonia ramosii* and *Begonia oxysperma*.

(a2) petiole	transverse section has one distinct furrow on the adaxial side	transverse section is circular
	uniseriate epidermis	uniseriate epidermis
	outer cortex (3 layers collenchymas cells)	outer cortex (4 to 6 layers collenchyma cells)
	inner cortex (2 to 5 layers of parenchyma cells)	inner cortex (3 to 8 layers inner parenchyma cells)
	vascular bundles arranged in a single ring	vascular bundles arranged in one or two rings
(a3) adventitious root	heavily stained with Fast green	heavily stained with Safranin O
	has 3 to 4 layers of small cortical cells	has 3 to 7 layers of large cortical cells
	open vascular bundles arranged in amphicribal manner	open vascular bundles arranged in amphicribal manner
(a4) leaf	vascular bundles arranged in a single ring along the midrib	vascular bundles arranged in a single ring along the midrib
	4 to 5 vascular bundles along the midrib	4 to 9 vascular bundles along the midrib
	uniseriate epidermis	uniseriate epidermis
	2 to 4 layers of collenchyma cells next to the epidermis	3 to 6 layers of collenchyma cells next to the epidermis
(a5) paradermal section	hypostomatic leaf	hypostomatic leaf
	anisocytic; surrounded by 3 irregularly-sized subsidiary cells	helicocytic; surrounded by 4 to 6 irregularly-sized subsidiary cells
	single stomata	cluster of stomata
	25 to 40 stomata per mm ²	23 to 74 stomata per mm ²
(b) reproductive structures		
(b1) carpellate flower	three-loculed and has axile placentation	three-loculed and has axile placentation
	uniseriate darkly-stained epidermal cells	uniseriate darkly-stained epidermal cells
	locule has large, short and bifurcating funiculi	locule has thin separated funiculi
(b2) staminate flower	tepals have uniseriate epidermis	not described due to limited number of samples
	3-5 layers of thin-walled cells between the outer and inner epidermis	
	anthers are with biseriate walls	

using 10 mL of 80% acetone as extraction solvent. An aliquot of the leaf extract was transferred in a cuvette and the absorbance reading at 645 nm and 663 nm was determined using UV-VIS spectrophotometer.

Measurement of environmental parameters

Environmental parameters such as elevation, soil and air temperatures, relative humidity and light intensity were measured in all sampling sites. The elevation was determined using Global Positioning System (GPS) while the soil and air temperature was measured using a thermometer. On the other hand, the relative humidity and light intensity were measured using a hygrometer and a light meter, respectively.

Statistical analysis

The correlation between quantitative characters and environmental parameters was determined using Pearson correlation. Microsoft Excel and SPSS Version 16. 0 were used for all the statistical analysis.

Results

The distributional range of *B. ramosii* and *B. oxysperma* in Mt. Banahaw de Lucban is very distinct. *B. ramosii* populations occur at an altitude of 742 to 880 masl, while *B. oxysperma* populations typically occur from 900 to 1115 masl. Two populations of *B. oxysperma* overlapped with *B. ramosii* populations at 780 to 860 masl (Figure 1). The chlorophyll *a* concentration varied between species and altitude however, no continuous variation was observed. *B. ramosii* had an average chlorophyll *a* concentration of 28.07 µg/mL while *B. oxysperma* had 11.52 µg/mL.

Morphology of vegetative and reproductive structures

The morphology of the vegetative and reproductive structures of *Begonia* spp. is summarized in Table 1. *Begonia ramosii* is a sub-shrub, which possessed erect, green or maroon stem. The presence and degree of anthocyanin pigmentation in the stem differ intraspecifically among *B. ramosii* populations (Figures 2A-D) while no variations were observed in other vegetative parts such as rhizome, petiole and leaf margin. Meanwhile, *B. oxysperma* is an epiphytic vine with succulent stem and no apparent anthocyanin pigmentation in any vegetative parts except for the lamina of young leaf (Figures 3A-B). The anthocyanin pigmentation became greatly reduced in mature leaves.

During the survey, only few individuals were observed to bear reproductive structures such as fruits and flowers. Both species are considered monoecious, bearing both staminate and

carpellate flowers on the same plant. The inflorescence of both species is axillary. *B. ramosii* was observed to possess dichasial cyme inflorescence while *B. oxysperma* had multiparous cymes. The carpellate flowers of *B. ramosii* were observed more often during the sampling period, and only infrequently in *B. oxysperma* because most of flowers of the latter had already developed into fruits. The carpellate flower of *B. ramosii* possesses five pink tepals with deep pink pigmentation at the base. The carpels of *B. ramosii* are actinomorphic, yellow, and have three styles associated with bifid spiraling stigmas (Figure 4A). Both species possessed three-winged inferior ovary. The staminate flower of *B. ramosii* has two tepals which are white with deep pink spot at the base (Figure 4B). In contrast, *B. oxysperma* has four tepals, with the outer pair being orange and the inner pair being pale orange (Figures 5A-B). The matured fruits of both species are dry dehiscent capsules with three wings. They are unequally sized having oblique wings larger than the lateral wings. The wings of the capsules of both species are turbinate in shape. The capsules of *B. ramosii* are pale pink-green (Figure 6A) while those of *B. oxysperma* are dark orange-brown (Figure 6B). The seed of *B. oxysperma* is dark brown, elongated (Figure 6C) and measures 0.12 to 0.20 mm in length. *B. ramosii* has also dark brown but rounded seeds measuring 0.06 to 0.10 mm (Figure 6D).

Anatomy of vegetative structures

The anatomical comparison of the vegetative structures of *Begonia ramosii* and *B. oxysperma* is presented in Table 1. Both species possessed a uniseriate epidermis. Multicellular branched and unbranched, non-glandular trichomes were also observed on the epidermal surface of the stem of *B. oxysperma* though their densities differed among populations. Most of the populations exhibited moderately dense trichomes while some populations exhibited extremely dense and scarce trichomes. Similarly, *B. ramosii* possessed minute multicellular, non-glandular trichomes.

The stem of *B. oxysperma* in transverse section is circular in shape (Figures 7A-B), while it contains ridges and furrows in *B. ramosii* (Figures 7E-F). An unusual structure known as medullary vascular bundles was observed in *B. oxysperma* in samples that occur in populations 11, 16 and 18 (Figure 7B). The cortex of *B. oxysperma* is differentiated into an internal and an external zone (Figure 7C). The external zone consists of 4-7 layers of collenchymatous cells while the internal zone consists of 5-15 layers of parenchymatous cells. Similarly, the cortex of *B. ramosii* is characterized by external zone of 3-5 layers of collenchymatous cells and an internal zone of 3-5 layers of parenchymatous cells (Figure 7F). The vascular system of both species is fascicular, with collateral vascular bundles arranged in

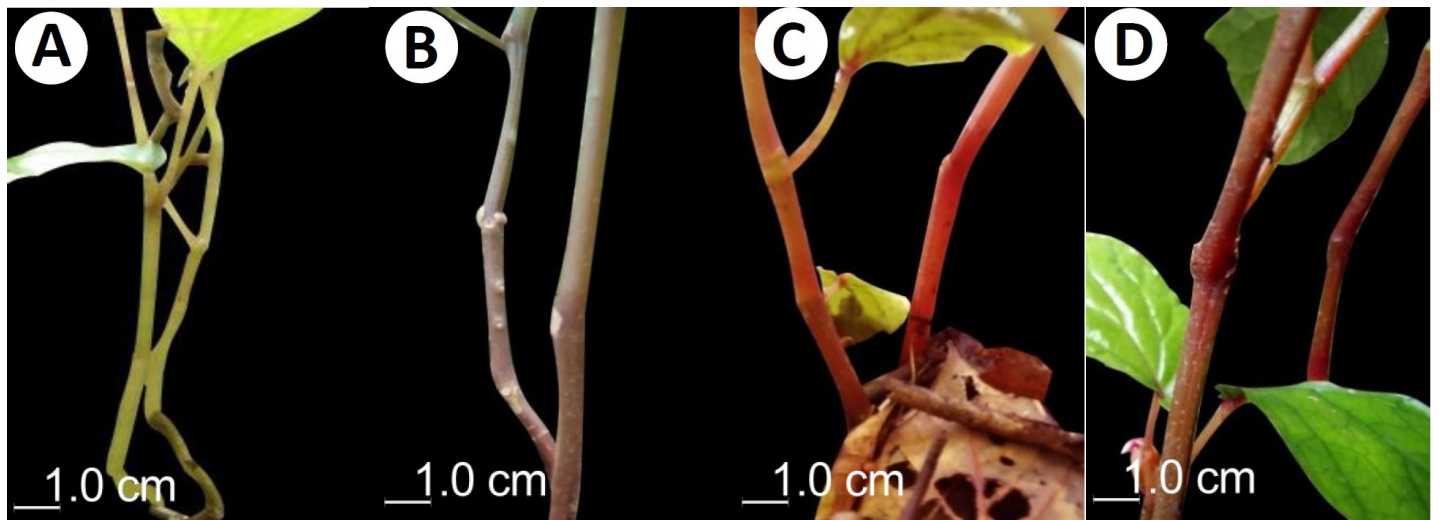


Figure 2. Variation in the degree of anthocyanin pigmentation of the stem of *Begonia ramosii*.



Figure 3. Variation in the degree of anthocyanin pigmentation on the leaves of *Begonia oxysperma*.

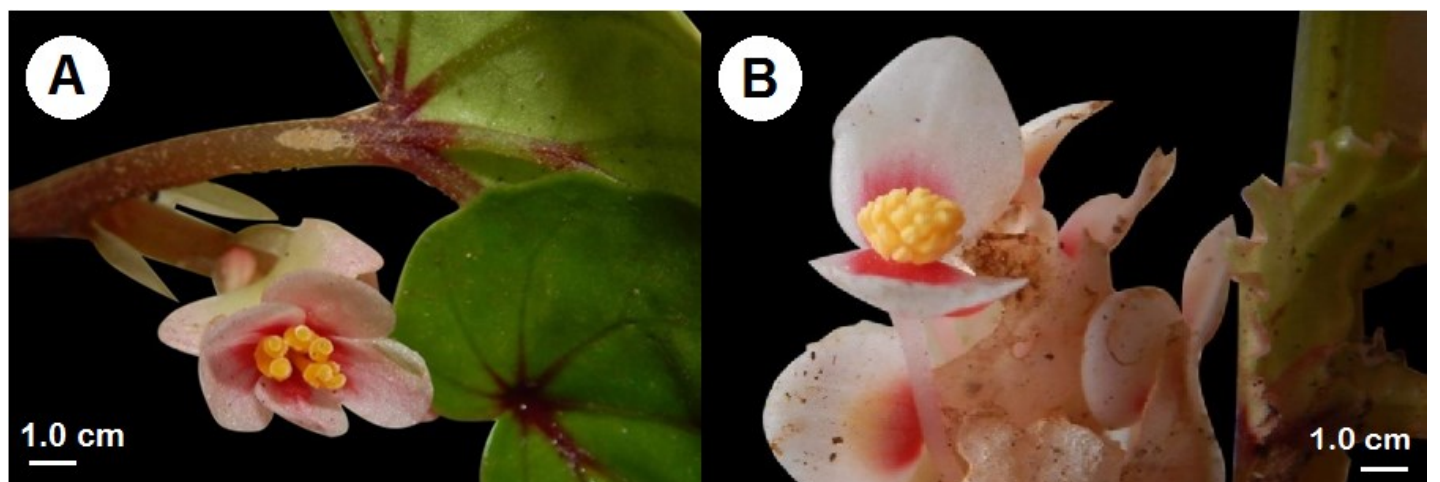


Figure 4. Carpellate flower (A) and staminate flower (B) of *Begonia ramosii*.

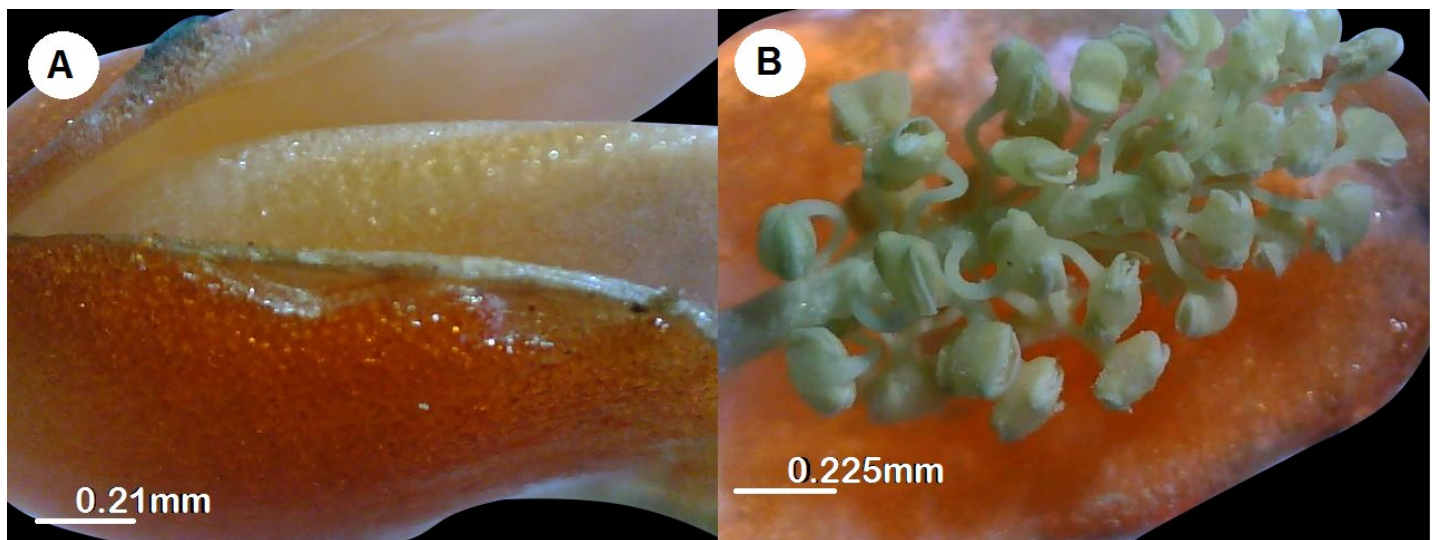


Figure 5. Staminate flower of *Begonia oxysperma*. (A) enlarged staminate flower showing the tepals; (B) enlarged flower showing the anthers.

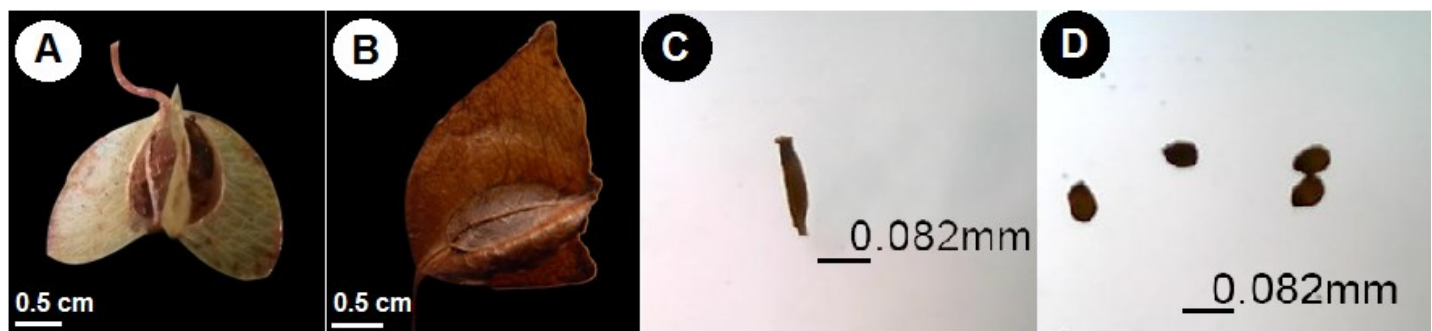


Figure 6. Fruit and seed of *Begonia ramosii* (A and D) and *Begonia oxysperma* (B and C).

a single ring. The interfascicular region is very prominent in *B. ramosii* and consists of purple, rectangular-shaped cells (Figure 7G), while the interfascicular region of *B. oxysperma* consists of a less prominent interfascicular cambium (Figure 7D).

The petiole of *B. oxysperma* consists of multicellular, non-glandular trichomes which extend densely from the base of the petiole up to the leaf base. On the other hand, the petiole of *B. ramosii* possesses evenly distributed minute trichomes. In the transverse section, the petiole of *B. oxysperma* is circular (Figure 8A) while *B. ramosii* has one distinct furrow on the adaxial side of the petiole (Figure 8C). Both species possess uniseriate epidermis. The cortex of *B. oxysperma* is composed of two distinct layers of basic tissues that include 4-6 layers of outer collenchyma and 3-8 layers of inner parenchyma (Figure 8B). Similarly, the cortex of *B. ramosii* consists of the same tissues but occur in fewer layers, having 3 layers of collenchymatous cells and 2-5 layers of parenchymatous cells (Figure 8D). The vascular bundles of *B. oxysperma* are arranged in one or two rings, with larger vascular bundles on the inner

ring and smaller on the outer ring. Fewer numbers of vascular bundles were observed on individuals that have *Cyathea* (tree fern) as their host plant while considerably more vascular bundles were observed on individuals that have dead or living woody trees as their host regardless of the population in which they belong. On the other hand, the vascular bundles of *B. ramosii* are arranged on a single ring of alternating large and small vascular bundles that range from 10 to 13.

The transverse sections of adventitious roots of *B. oxysperma* and *B. ramosii* are circular. *B. oxysperma* roots were heavily stained with Safranin O (Figure 9A) whereas *B. ramosii* were heavily stained with Fast green (Figure 9C). Both species exhibit periderm which covers the inner cortex and vascular cylinders. *B. oxysperma* has 3-7 layers of large cortical cells while *B. ramosii* has 3-4 layers of comparably smaller cortical cells. The vascular bundles of both species are open, possessing secondary growth and arranged in an amphicribal manner, with the phloem being held external to the xylem (Figures 9B & D).

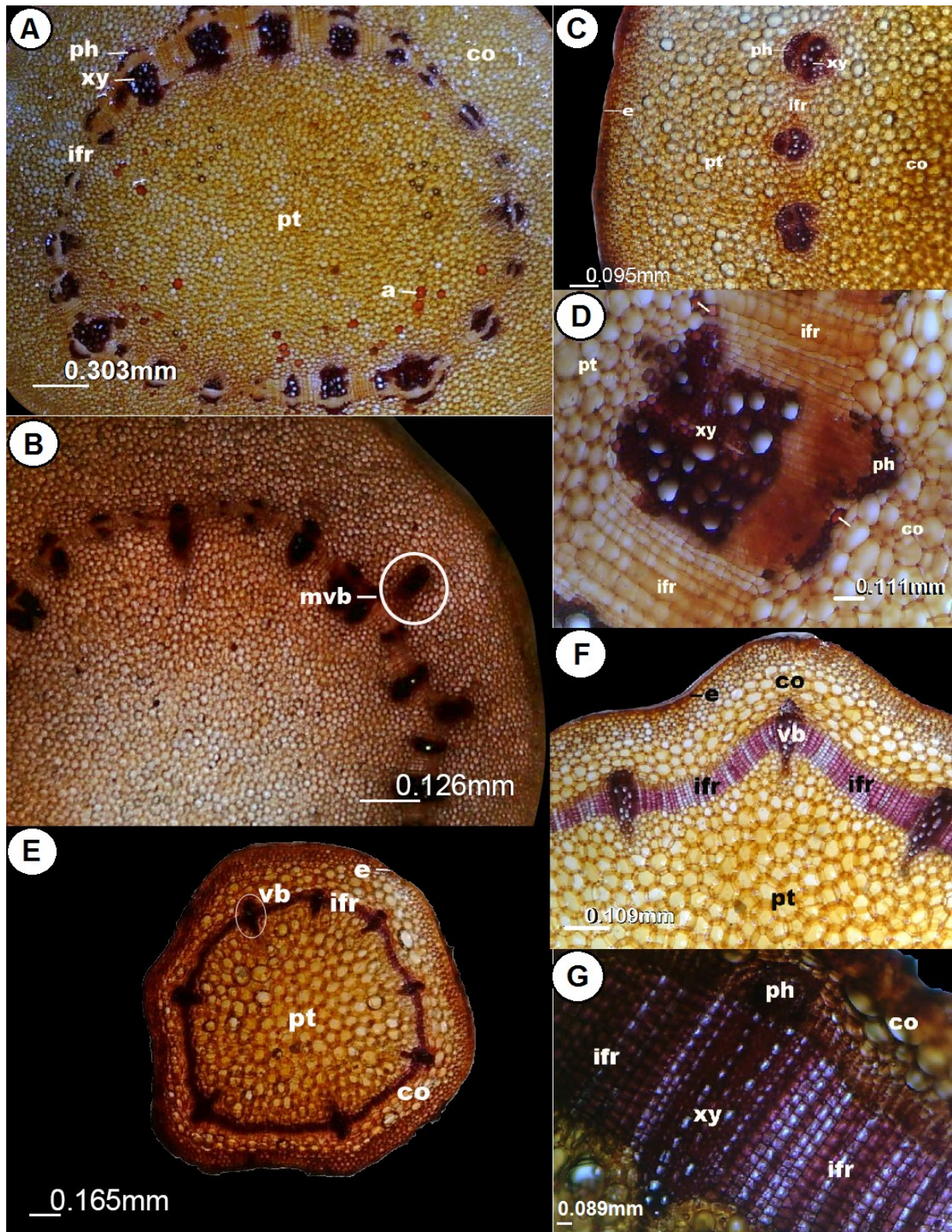


Figure 7. Transverse section of the stem of *Begonia oxysperma* (A-D) and *Begonia ramosii* (E-G). Abbreviations—ph: phloem; xy: xylem; co: cortex; pt: pith; a: phenolic compound deposition; e: epidermis; ifr: interfascicular region; mvb: medullary vascular bundle.

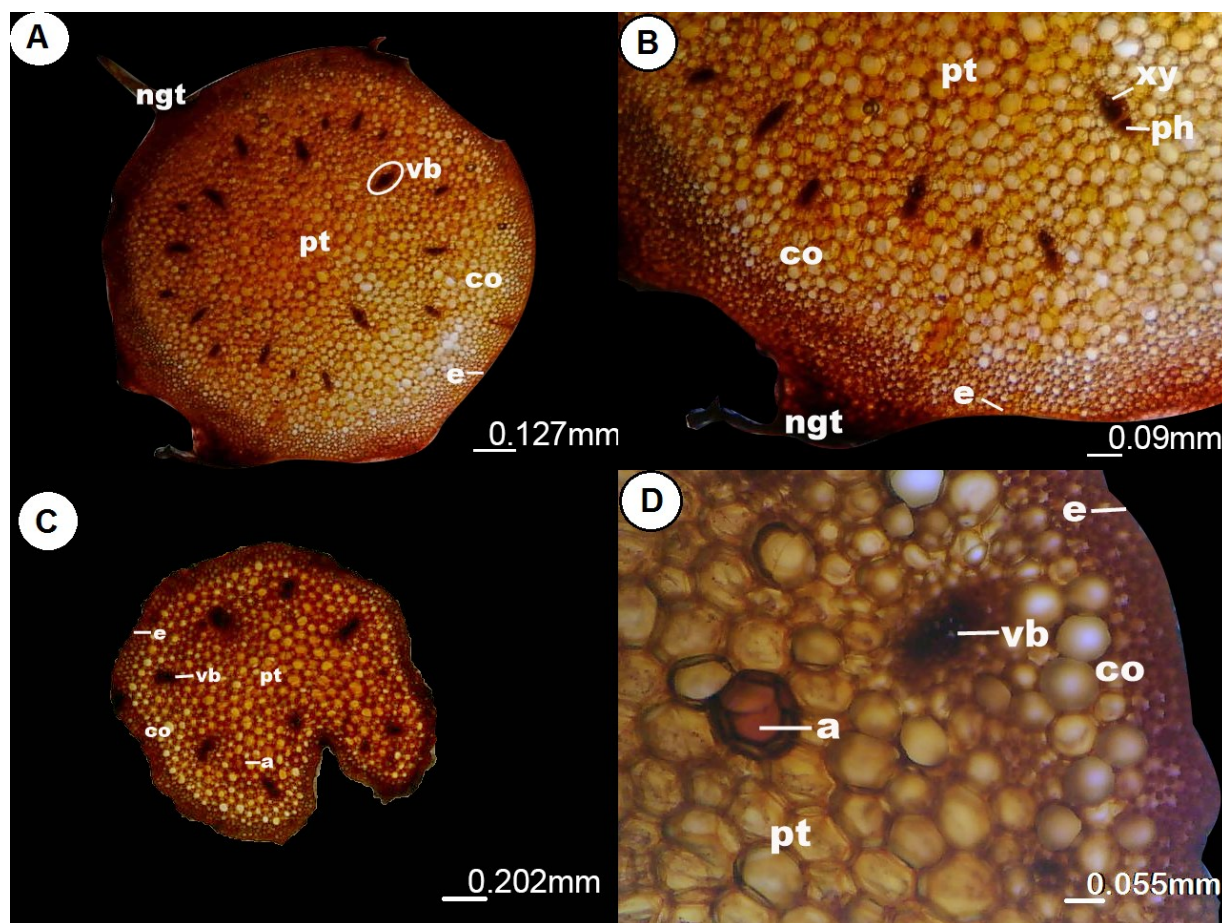


Figure 8. Transverse section of the petiole of *Begonia oxysperma* (A-B) and *Begonia ramosii* (C-D). Abbreviations—ngt: non-glandular trichome; vb: vascular bundle; pt: pith; co: cortex; e: epidermis; xy: xylem; ph: phloem; a: phenolic compound deposition.

The leaves of *B. oxysperma* are generally thicker than those of *B. ramosii* while the vascular bundles of both species are arranged in a single ring along the midrib. *B. oxysperma* populations possessed 4 to 9 vascular bundles along the midrib while *B. ramosii* possessed 4 to 5 vascular bundles along the midrib. Both species possessed uniseriate epidermis. Along the midrib, next to the epidermis are 3-6 layers of collenchymatous cells in *B. oxysperma* while there are 2-4 layers of collenchymatous cells in *B. ramosii*. Parenchymatous cells mostly comprise the ground tissue of the midrib of both species. The leaf laminar portion of *B. oxysperma* is characterized by large cells while that of *B. ramosii* are relatively smaller and thinner. Next to the epidermis on the adaxial surface of *B. oxysperma* are 1-2 layers of palisade mesophyll cells. The palisade mesophyll cells are mostly elongated cells that are tightly packed. On the abaxial surface, spongy mesophyll cells are found which are somewhat spherical in shape.

Paradermal sections showed that both species possessed a hypostomatic leaf, with stomata present on the abaxial leaf

surface but absent on the adaxial leaf surface. The stomata of *B. oxysperma* are helicocytic, being helically surrounded by 4 to 6 irregularly-sized subsidiary cells (Figure 10A) while those of *B. ramosii* is anisocytic, being surrounded by 3 irregularly-sized subsidiary cells (Figure 10B). Though both hypostomatic, the stomata of the two species differ in distribution. *B. ramosii* possesses singly occurring stomata while *B. oxysperma* has stomata that occur in clusters. The stomatal density in *B. ramosii* ranges from 25 to 40 per mm² though it varies among populations. Meanwhile, the stomatal density of *B. oxysperma* ranges from 23 to 74 stomata per mm². Higher stomatal density in *B. oxysperma* might contribute to water-use efficiency and regulation of gas exchange. *B. oxysperma* possesses multicellular, non-glandular trichomes on the leaf epidermal surface. Trichomes were observed mainly on the primary leaf veins while others were observed along the leaf margin and lamina at varying densities. *B. ramosii* populations also possess minute multicellular, non-glandular trichomes, which were also confined to the leaf veins.

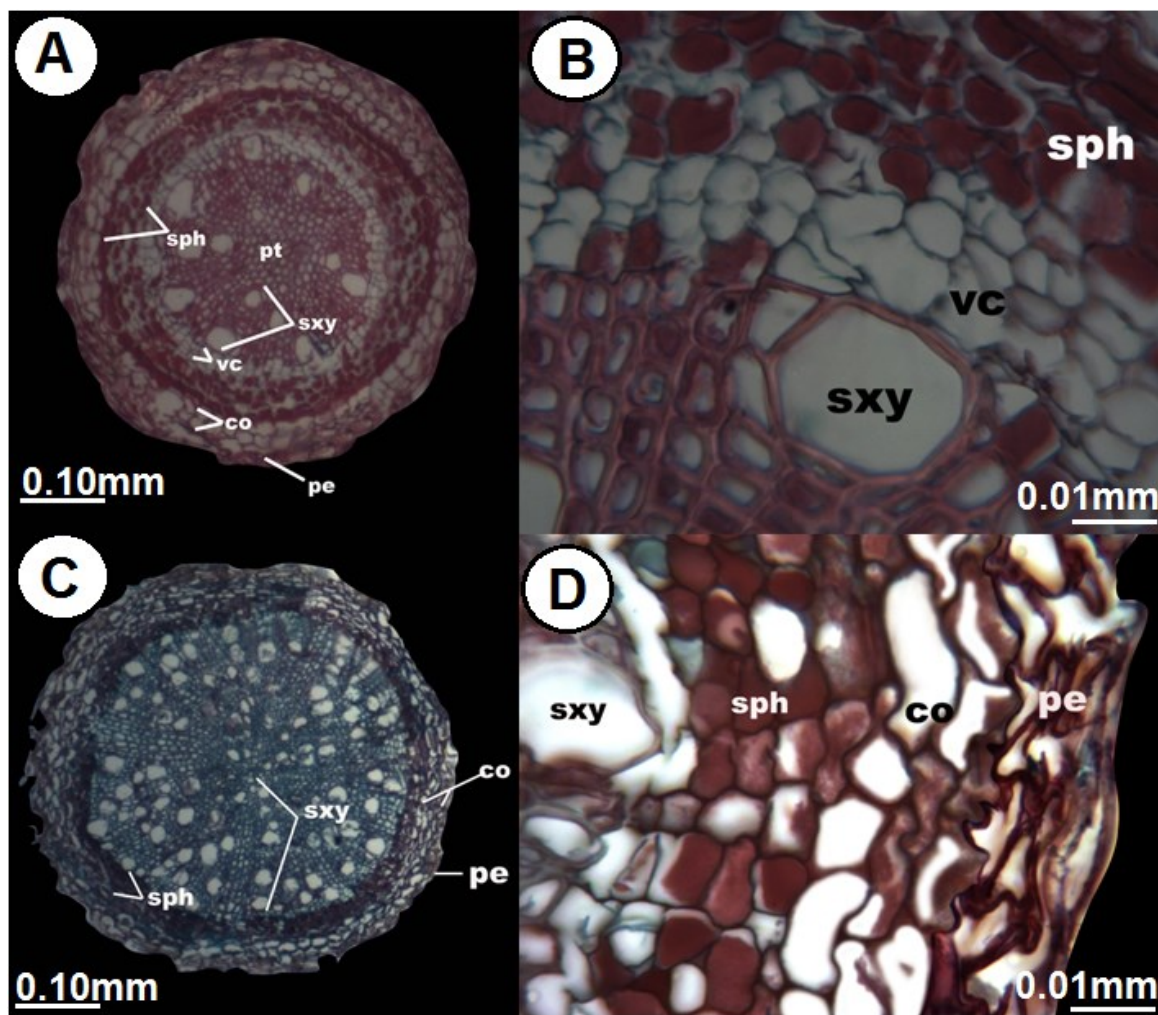


Figure 9. Transverse section of the adventitious root of *Begonia oxysperma* (A-B) and *Begonia ramosii* (C-D). Abbreviations—sph: secondary phloem; pt: pith; sxy: secondary xylem; vc: vascular cambium; pe: periderm; co: cortex.

Anatomy of reproductive structures

The carpellate flowers of both species are three-loculed and exhibit axile placentation. The ovary walls of both species have uniseriate darkly-stained epidermal cells. Each locule of *B. oxysperma* has thin separated funiculi (Figure 11A) while *B. ramosii* has large, short and bifurcating funiculi (Figure 11B). The staminate flower of *B. ramosii* possesses tepals which have uniseriate epidermis (Figure 11C). The epidermal cells on the outer surface of the tepals are larger than those found on the inner surface (Figure 11D). In between the outer and inner epidermal cells are 3-5 layers of thin-walled cells. The anthers are tetrasporangiate and have biseriate walls (Figure 11E).

Discussion

The distribution of *B. oxysperma* and *B. ramosii* populations overlapped at a certain point, but beyond that, *B. ramosii* was clearly replaced by *B. oxysperma* populations at higher elevations. Thus, they are regarded as ecological specialists, each being found only in a narrow altitudinal range (Griffith & Sultan, 2012). The first functional trait observed was the anthocyanin pigmentation on the lamina of young leaves of *B. oxysperma* and vegetative structures of *B. ramosii* which might be adaptive for providing protection against the damaging effects of sunlight while they are still in the process of growth and development of their photosynthetic leaf cells (Cutuli *et al.*, 1999; Guo *et al.*, 2008). We classified anthocyanin pigmentation as a localized phenotype being more intense in areas with low

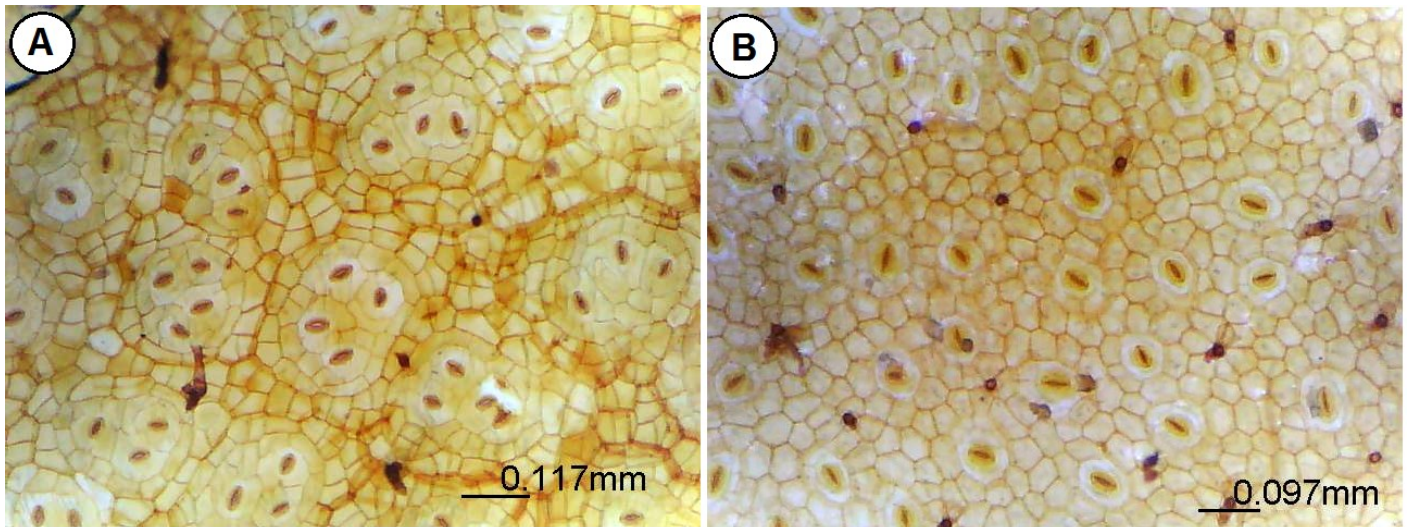


Figure 10. Paradermal section of *Begonia oxysperma* (A) and *Begonia ramosii* (B).

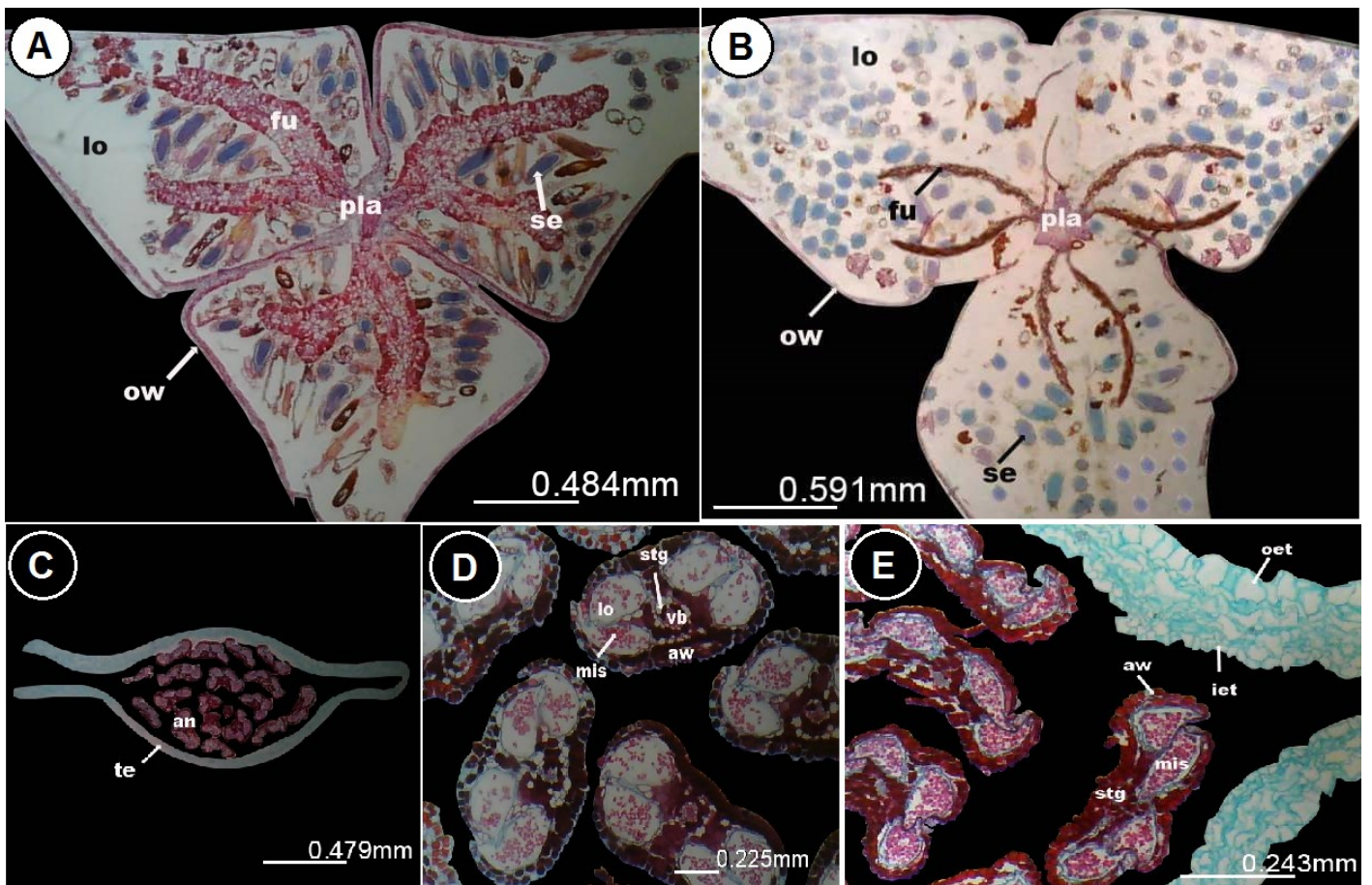


Figure 11. Transverse section of the carpellate flower of *Begonia oxysperma* (A) and *Begonia ramosii* (B) and staminate flower of *Begonia ramosii* (C-E). Abbreviations—an: anther; te: tepal; lo: locule; stg: stigma; vb: vascular bundle; mis: microsporangiospores; aw: anther wall; iet: inner epidermis of tepal; oet: outer epidermis of tepal; pla: placenta; fu: funiculus; ow: ovary wall; se: seed.

temperature. Using transcriptome analysis, Dong *et al.* (2018) found that anthocyanin accumulation was employed as a common strategy by *Begonia* seedlings in resisting low temperature and high light stress. However, we found no significant correlation between light intensity and degree of anthocyanin pigmentation ($p > 0.05$).

The seeds of both species are very small, light and numerous which serve as an adaptation for wind dispersal (anemochory). When matured enough to produce seeds, capsules of both species turn brown and split between the locule and wing (Rubite, 2013). The seed and fruit characteristics of both species confer a wide range of dispersal. In our study, we found a positive correlation between average fruit length ($r = 0.266$; $p = 0.002$) and total number of fruits ($r = 0.266$; $p = 0.002$) with light intensity. Several studies (Jeong *et al.*, 2009; Kessler & Armitage, 1992) reported that begonias tend to have smaller and fewer flowers and fruits in the absence of adequate light. This only suggests that optimal light intensity to induce flowering and fruiting differs among *Begonia* species. Populations of *Begonia* spp. that are close to each other showed similar average fruit length and fruiting period which suggests localization of phenotypes. Functional traits are predictors of plants in response to environmental stimuli. Mt. Banahaw creates a micro-climate that allows the *Begonia* to develop functional characters. The presence of non-glandular trichomes provides a microlayer around the plant tissue in response to high relative humidity (Georgiev & Naydenova, 2013). Thus, *Begonia* populations occurring on humid environments tend to possess lower trichome density while those with habitat of lower relative humidity tend to have higher trichome density.

The number of layers of collenchymatous cells on the cortex of stems for both species functions mainly for water storage and support. The presence of large amount of pectin on cell wall of collenchymatous cells makes them hydrophilic, allowing them to store water while the presence of cellulose and hemicellulose provide structural support (Jarvis & Apperly, 1990). Meanwhile, the layer of parenchymatous cells in the cortex of *B. oxysperma* was relatively thicker than that of *B. ramosii*. This might be due to adaptation of the epiphytic *B. oxysperma* to conserve water intracellularly within the cortex. These parenchymatous cells are able to store water due to its thin wall and large air spaces, which are capable of elastic water storage (Borchert, 1994). The central pith region of both species also consisted mainly of parenchymatous cells which also function primarily for storage of water, mineral and other nutrients. Several populations of *B. oxysperma* including populations 6, 8, 10, 11 and 12 were observed to possess red-stained cells near the vascular bundles at the pith region suggesting that it is a localized phenotype. This might be due to

the presence of phenolic vacuolar inclusions (Gutmann, 1995). These phenolic compounds might contribute to resistance of *B. oxysperma* against herbivory and against the action of other parasitic microorganisms. Furthermore, these phenolic compounds might be a candidate component for possible antioxidant and antimicrobial property as in other related *Begonia* species (Isaivani *et al.*, 2014; Jeeva & Johnson, 2012; Kalpanadevi & Mohan, 2012). The host plant of *B. oxysperma* might contribute to the formation of medullary vascular bundles. Dead woody trees provide drier environment to epiphytic *B. oxysperma*. As an adaptive mechanism, medullary cells develop on the large pith, allowing the cells along the pith to store water and starch (Mauseth, 1993). In our study involving 76 individual samples of *B. oxysperma*, 36 had *Cyathea* as their host, 9 had dead tree, 2 had *Pandanus* and the remaining 29 had a live woody tree as their host plant. Our results suggested that host tree with larger diameter provide greater substrate area and considerably greater amount of organic materials that epiphytic *B. oxysperma* can utilize. The presence of vascular cambium contributed to the secondary growth and rigidity of the stem of both species which serve primarily for support. Red-stained cells similar to those found on the leaves and stem of *B. oxysperma* were also observed on the transverse section of the petiole of *B. ramosii* due to the presence of phenolic compound which offers protection against herbivory.

The degree of histochemical stain absorption in the roots suggests that the main compound present in the cell walls of *B. oxysperma* is lignin, while in *B. ramosii* it is cellulose. The large amount of lignin present in between the cell walls of *B. oxysperma* cells serves as an adaptation among most epiphytes for water and ion regulation in apoplastic route (Dickison, 2000). The cells of the periderm of both species are both darkly stained due to the presence of suberin, which acts as chemical barrier to limit the extracellular and apoplastic transport of water and solutes in plant roots (Baxter *et al.*, 2009). However, *B. oxysperma* was found to possess a thicker periderm compared to *B. ramosii*. This might serve as an adaptation to increase mechanical protection and prevent water loss, as occurs in most epiphytes (Moreira *et al.*, 2008; Nawaz *et al.*, 2013). Associated with the periderm of *B. oxysperma* are large, elongated parenchymatous cells, which comprise the cortex and potentially serve as a water reservoir.

The secondary phloem of root sections for both species was conspicuous due to red-stained cells which might be due to the presence of organic compounds specifically carbohydrates in the internal portion of the cells. Meanwhile, the xylem vessels of both species possessed thickened cell walls which provide maximum structural support by preventing them from collapsing in case of extreme root pressure. The diameter of xylem vessels

of *B. ramosii* was found to be smaller compared to that of *B. oxysperma*, but occurred in higher frequency. Holste *et al.* (2006) observed that conduit diameters of xylem tend to be reduced and its number increases when subjected to environments with decreased water supply. Changes in the conduit diameter dramatically affect the hydraulic conductivity of roots. Thus, xylem vessels with smaller diameter would have lower hydraulic conductivity than those with larger diameter. To compensate for this, *B. ramosii* xylem vessels occurred in greater number. *B. oxysperma*, on the other hand, had xylem vessels with larger diameter but fewer in number.

Transverse sections of the leaves of *B. oxysperma* were generally thicker than *B. ramosii* which serve as an adaptation to exposure to full sunlight being an epiphyte. According to Kubinova (1991), leaves exposed to high light intensity generally develop multiple cell layers. Evans (1999) pointed out that the increase in total lamina thickness is mainly due to the greater palisade parenchyma, spongy parenchyma, and epidermal tissue thickness. *B. oxysperma* possessed non-contiguous stomatal clusters having groups of stomata that are separated by subsidiary cells. Each stomatal cluster consisted of 2 to 6 stomata. According to Hoover (1986), stomatal clustering serves as an adaptation for more efficient conservation of water. The density as well as distribution of trichomes along the leaf surface was observed to differ among *B. oxysperma* populations. Our results showed a positive correlation of stomatal density with altitude ($r = 0.479$; $p < 0.01$) and light intensity ($r = 0.412$; $p = 0.003$). We observed low stomatal density in areas with low light intensity suggesting that it is a localized phenotype. Together with other epidermal cells, trichomes determine the rate of epidermal transpiration and energy allocation on the leaves. According to Hall *et al.* (1993), non-glandular trichomes on leaf lamina reflect UV-B for photoprotection which could be the reason why most populations generally possess scarce trichome density on leaf abaxial surface and moderately dense trichomes on the adaxial side.

Conclusions and Recommendation

We have reported in this paper the functional traits of *B. oxysperma* and *B. ramosii* along the altitudinal gradients of Mt. Banahaw de Lucban using morpho-anatomical data. Even though the technique is relatively simple, we have provided evidences of how the environment shaped the morphology and anatomy of plants specifically *Begonia*. The two species are relatively similar in terms of their morpho-anatomy but some functional traits unique for each species were observed which confer adaptation. Our preliminary results are very important to

understand the ecological adaptations of *Begonia* especially nowadays that we are experiencing climatic changes. Further explorations of wild *Begonia* populations found in other peaks of the Mt. Banahaw-San Cristobal Protected Landscape can be done to deliver a more extensive comparison of the *Begonia* species in the area. A study at the molecular level may also provide a more thorough analysis of the phenotypic variations among these *Begonia* species.

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The authors declared no conflict of interest or any financial interest or benefit that has arisen from the direct applications of this research.

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